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Effects of sunrise/sunset lighting on corticosterone levels in Coturnix quail (Coturnix coturnix)

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Honors Thesis

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Abstract

Both genetics and environment play an important role in the growth, performance and overall welfare of poultry species. Current commercial production practices typically do not mimic the natural environmental conditions of ancestral poultry species, specifically lighting requirements. In nature, poultry species are subjected to natural day length and the slow rising and setting of the sun. This is compared to commercial conditions in which lights are sudden on/off, not mimicking the natural trajectory of the sun in terms of light intensity and exposure. The current study aimed to evaluate the impact of genetics and the effect of sunrise/sunset lighting on the stress response of 4 genetic lines of coturnix quail. The 4 lines utilized in this study include a high stress (H), low stress (L), stress control (R), and Arkansas randombred (A) quail lines. Day old chicks from the lines were placed in one of two environmentally controlled rooms. All conditions were kept similar between the rooms until week 4. At week 4, half of the quail in each room were relocated to the other room and the experimental conditions began. One room was subjected to sudden on/off lighting while the other room was subjected to a 1-hour long sunrise/sunset treatment. At 8 weeks of age, blood was collected from each line of quail at 5 time points: before lights on, 3 points during sunrise lighting, and after lights on at full intensity. The subsequent serum samples were then evaluated for their respective corticosterone levels. The results of this study show that the sunrise/sunset lighting room shows lower corticosterone levels overall and transferred quail have had elevated corticosterone levels, indicating they were under stress. These results lead to the conclusion that sunrise/sunset lighting has the potential to improve overall production, regardless of genetic line.

Keywords: *Coturnix coturnix*; lighting; sunrise/sunset; corticosterone

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Chapter 1. Introduction

Background and Need

Poultry species including chickens, turkeys, and quail are typically grown in controlled environments to maximize both genetic potential and economic gains. In these controlled environments, nearly every aspect of management, including temperature, humidity, day length, light intensity, water pressure, and diet is regulated. Characterizing the best environment to grow poultry is dynamic since the commercial bird is constantly changing due to genetic selection. Therefore, the rearing environment must change in order to identify the environment that will maximize genetic potential. As mentioned, lighting is controlled during production but fails to emulate the natural habitat from which their ancestors evolved (Manci et al., 2011). Wild bird habitat is consistent with tall grass environments such as agricultural fields and grasslands all over Europe, Asia, and the southern United States (Wetherbee, 1961). The environment is not controlled.

Current production practices continue to utilize traditional on/off lighting, though the technology for gradual sunrise/sunset lighting is available (Manci et al., 2011). Hypothesized benefits of the gradual lighting system include the creation of a low stress environment as indicated by reduced corticosterone (Majer et al., 2019). This leads to increased growth rate, better metabolism, improved overall bird welfare through a lessened stress response.

In North America, natural day length is not practical for sustained commercial egg production. Light supplementation that is balanced between natural and artificial day length is necessary to maintain peak production (Rubinoff, 2016) and persistence of lay. For confinement housing with solid sidewalls, artificial lighting is the only light available. Synchronization of lay can be easily measured by tracking a hens daily production record. Achieving synchrony of lay is

important for consistent performance of the birds in a poultry house. Since breeder management changes with age of the bird, it is important to establish and maintain flock uniformity by managing nutrition and lighting requirements. It is easier to manage a uniform flock because you will not need to provide special attention to small or oversized breeders.

Problem Statement

Current research has focused on the effects of light intensity and spectrum and its impact on hormone production, metabolism, and other aspects of avian physiology and reproduction (Ibrahim et al., 2012), but there is minimal research investigating how lights turn on and off and the effect on hormonal stress response, specifically through levels of corticosterone.

The physiological effects of traditional on/off lighting are not known. Most research has focused on light spectrums and light intensity rather than how the abrupt lights on and off affects the bird. One study focusing on gradual lighting on fish was found (Ryu et al., 2019). Ryu and colleagues (2019) determined that plasma levels of cortisol were significantly higher in the control group when compared to the group subjected to dimming lighting. Evaluating the impacts of light intensity changes on birds will help advance the optimal environmental conditions required for maximized poultry production and improved bird welfare.

Purpose Statement

The purpose of this experiment is to determine the impact of gradual sunrise/sunset lighting on hormonal stress levels in quail as compared to traditional on/off lighting. In addition, lines of quail, known to differ in stress response, will be exposed to the various lighting treatments to evaluate potential genotype by environment response.

Research Objective

The main objective of this experiment was to evaluate the degree to which traditional on/off, sudden changes in light availability impact physiological stress indicators when compared to gradual changes in light intensity through sunrise/sunset lighting.

Hypothesis

The hypothesis for this study is that the quail in the experimental sunrise/sunset lighting system will have lower corticosterone levels than the control on/off lighting system. The sunrise/sunset lighting system will better mimic what their ancestors would be exposed to, and that the control group will be less synchronized than the experimental group.

Chapter 2. Literature Review

Quail

Coturnix quail are a popular choice in both genetic and environmental research due to their short generation interval, rapid growth rate, and small size. If managed properly, quail can reach sexual maturity as early as 6 weeks of age, allowing multiple replications of selection studies in a short amount of time, compared to chickens, who reach maturity at 20 to 24 weeks of age. The short generation interval for quail makes them an ideal choice for selection programs where time is limited.

Coturnix coturnix japonica originated in Europe and Asia as a migrating bird, though their migration patterns are not known. They were introduced to North America from Italy and other countries starting in 1877 and were established in the southern United States by the 1950s (Wetherbee, 1961). In the wild, Coturnix quail prefer to live in or near grasslands and agricultural fields and eat approximately 48 percent animal matter and 52 percent seeds (Wetherbee, 1961). Throughout the years, many researchers have utilized Japanese quail for selection programs. Specifically, lines of Coturnix quail utilized in these studies have been selected for corticosterone response when exposed to handling stress (Satterlee & Johnson, 1978).

Lighting requirements

Quail have different light requirements than humans. Poultry specifically are able to detect and require a larger spectrum of light than people. While humans have only one spectral peak around 550 to 560 nanometers (nm), poultry have three peaks at 480, 560, and 625 nm (Rubinoff, 2016). While the light intensity around 560 nm is considered acceptable, the lux at 480 and 625 nm might not be as bright, which is why a broad-spectrum bulb and an animal

specific light meter are important (Rubinoff, 2016). Ibrahim and colleagues (2012) concluded that different wavelengths of light target certain behaviors. They determined that birds raised under red light (peak at 625 nm) were more engaged in physical activity, eating, and pecking, while birds raised under blue and green lights (peaks 560 and 480 nm) exhibited higher weight, lower cortisol, but higher cholesterol levels (Ibrahim et al., 2012).

Photoperiod, the length of time of exposure to light, is important for the development of mature follicles and the reproductive tract and sustained production. Geng and colleagues (2018) found that the feed to egg ratio and average feed intake was optimal in birds with 16 hours of continuous light, while birds raised with 12 hours of light were the least efficient. The lighting method used for this study was a gradual activation and deactivation circuit, much like the circuit described by Manci and coworkers (1992).

Corticosterone

Corticosterone, also known as the stress hormone, is described to have negative effects on the growth, health, and welfare of poultry species. Hull and colleagues (2007) demonstrated this by administering quail with corticosterone in their water. The results of this study showed that peritoneal fat deposition increased, while muscle and organ weights decreased with increased corticosterone levels (Hull et al. 2007). In another experiment where quail were also administered corticosterone in water, corticosterone was found to induce stress, measured by reactive oxygen metabolites, whether or not there was a stressor present (Majer et al., 2019). The problem with these experiments is that quail were dosed at a certain concentration in the water, but different birds drink different amounts of water resulting in variable corticosterone intakes among the birds. The genetic selection for corticosterone response was necessary to truly understand the interplay of genetics and corticosterone in the stress response of birds. In a

selection study, two diverging lines and a control line were bred for twelve generations (Satterlee & Johnson, 1978). Briefly, Japanese quail were subjected to a brief mechanical stress in which they were restrained for a period of time. After the restraint period, plasma samples from the quail were evaluated for their stress response through corticosterone levels. From the base population line, two lines were diverged for stress response based on either high or low corticosterone levels. This study resulted in a high stress line, a low stress line, and a control line from which the selected lines were developed.

Corticosterone also affects behavior in poultry species. In a study, chicks considered "high stress" froze more, had less activity, and took longer to move around than the low stress chicks (Jones et al., 1992). This study also showed that the low stress line chicks explored more sectors of the field than the high stress chicks (Jones et al., 1992).

Ryu et al. (2019) conducted a similar study. This study evaluated the effects of a light dimming system on the corticosterone responses of fish. Fish were subjected to either a complete lights on/off control system or an experimental system in which lights were dimmed. The results showed that although both systems resulted in stress responses, the control group had levels of corticosterone two times higher than the experimental group that were subjected to the dimming lights (Ryu et al., 2019).

Chapter 3. Materials and Methods

Research Design

True experimental studies have a control group and one or more experimental groups (Gribbons & Herman, 1996). Data in a true experimental design is also quantitative, fitting the main objectives of this study. Four genetic lines of quail were utilized in this study. Two lines utilized included the high stress (H) and low stress (L), which were previously selected for corticosterone levels post mechanical restraint. The remaining two lines include a random bred control population (R) serving as the base population for the H and L lines and a second random bred line developed at the University of Arkansas known as the AR random (A). At hatch, straight run chicks from each of the 4 lines were equally and randomly divided into one of two rooms and subjected to either traditional sudden on/off lighting (Room 1) or gradual sunrise/sunset lighting (Room 2). At 4 weeks, a subset of birds from each room were switched between rooms. This created 4 environmental treatments. The environmental treatments include R1-N for chicks placed initially in R1 and not transferred, R1-T for chicks initially placed in room 1 but transferred to room 2, R2-N for chicks initially placed in room 2 but not transferred and R2-T for chicks placed in room 2 and transferred to room 1. At eight weeks, blood samples from all lines and treatment combinations were taken at 5 different time points (lights on, post lights on and additional time points for room 2, 3 points during the gradual sunrise lighting).

Population and Sampling

Four genetic lines of quail were used for this study. These included high stress (H), low stress (L), random (R), and Arkansas random (A). The H, L and R lines were originally selected as described in Satterlee and Johnson (1978) and have been maintained as random bred

populations at the University of Arkansas since the 1990s. The A line was developed as a random bred control of quail by Dr. Nick Anthony.

The sampling method used in this study was stratified sampling (Imbens, 1996). The quail were stratified by genetic line. At hatch, each chick was tagged with an identifying wing band with genetic line information through a corresponding number. On day of placement, each genetic line was split evenly into one of two rooms. One room was subjected to traditional sudden on, sudden off lighting while the second room was subjected to sunrise and sunset lighting at lights on and lights off. In the sunrise/sunset room, lights were programmed to come on gradually over a 1-hour period and shut off gradually over a 1-hour period, mimicking the natural rising and setting of the sun. On the day of placement, chicks received 24 h of light. From d 2 to d 7, the lighting schedule was reduced to 23 h light, 1 h dark. From d 8 on, lighting in both rooms was reduced to 18 h light, 6 h of dark. This lighting schedule remained in effect until sampling. At eight weeks, quail from the control and transfer to control were sampled before and after the lights turned on. Birds regardless of gender from the experimental and transfer to experimental were sampled before the lights turned on, every 20 minutes into room 2 lights turning on, and after the lights were fully on.

All quail were placed in fresh, pine shaving litter floor pens. Both rooms were located in the same research house. Supplemental waters were utilized for the first week after placement and water was provided ad libitum through a hanging nipple drinker water line. Feed was kept consistent for all treatments and a common commercial quail starter feed was provided ad libitum throughout the experiment period. Temperature and ventilation for both rooms were adjusted according to bird age.

Rigor

Steps used to combat mistakes are the quantitative and scientific methods followed for the study. All factors except how the lights turn on were kept the same; samples were randomly collected from a large group. This study was quantitative, and procedures kept all factors and data collection consistent. Controlled variables include diet, water, temperature, humidity, length of light, and light intensity.

Instrumentation

Instrumentation used in this study was a commercially available serum corticosterone enzyme linked immunosorbent assay (ELISA) kit (Enzo Life Sciences, Inc,) and a microplate reader. All samples were run in duplicate. Absorbance was measured at 405 nm (Corticosterone ELISA, n.d.).

Corticosterone Data Collection

To measure corticosterone levels of the birds, blood samples were collected into heparinized tubes and centrifuged to separate blood serum. For blood serum samples, a single bird from each line, lighting program and time of day was sampled. Blood sampling occurred post euthanization. Due to low hatch numbers in two of the genetic lines, sample size for this project was low and additional birds per time point could not be sampled. Blood samples were collected at 8 weeks of age once birds had been in production for 2 weeks. For the sampling procedure, quail were euthanized by cervical dislocation, then decapitated, and several mL of blood was collected into a heparinized tube. The blood was then centrifuged, and serum separated from the red blood cells and white blood cells. Serum samples were then frozen for evaluation of corticosterone at a later date.

Since this study involved the use of vertebrae animals, IACUC and AUP were submitted prior to the initiation of this study (Approval #18083-General Rearing of Selected Chicken and Quail Populations).

Data Analysis

As a result of low hatch numbers in the quail lines, multiple samples per time point was not possible. Therefore, statistical analysis could not be conducted however general comparisons are made.

Results and Discussion

Corticosterone levels from all samples collected are presented in Table 1. The remaining results aim to make comparisons based on relationships involving room, line, time, and transfer status conducted during the study period.

Room

Rooms varied based on the methods of lights on and off. Results of this study indicated that room 1, which was subjected to sudden on/off lighting had higher corticosterone levels overall than room 2 which was subjected to sunrise/sunset lighting (Figure 1). Sunrise/sunset lighting better mimics the lighting conditions a quail would receive in the wild. Although these quail lines have been continuously raised and selected in a situation where lights are sudden on and sudden off, having an environment that better mimics natural lighting that a quail would be subjected to in the wild appears to lower stress and has the potential to improve animal welfare conditions. These results are similar to the findings of Ryu and colleagues' fish study and dimming lights (2019).

Quail transferred between rooms at 4 weeks, regardless of line or treatment, had higher corticosterone levels than quail that remained in the same treatment throughout the study (Figure 2). Transfer between rooms represents an additional stress. Even though quail were allowed to acclimate to the new rooms for 4 weeks, the period may have been too short to overcome the negative impacts of the transfer stress.

Line

Overall averages for all samples collected from birds of each of the lines are displayed in figure 7. Interestingly, the L line had the highest corticosterone levels when compared to the other populations. However, when line averages are broken out by time collected, it appears that

in the dark, prior to lights on, the H line had a relatively low corticosterone level that slowly increased as the lighting reached full intensity. The remaining 3 lines had higher corticosterone levels prior to lights coming on that appeared to decrease with low light intensities. As light intensity increased, so did corticosterone levels for lines R, L and A. Line A and R had corticosterone levels higher than H and L before the lights turned on, then settled between H and L while the lights were turning on, then were the lowest of all bloodlines once the lights were at full intensity. The H and L lines were originally selected for their corticosterone levels after a brief mechanical stress was applied to them (Satterlee and Johnson, 1978). It is possible the H line has a lower basal level of corticosterone as observed by the low corticosterone level exhibited by the H line quail during the dark period, however when a stress or change in environment is applied to the quail, a sudden but drastic increase in circulating corticosterone levels can be observed.

While generally, transferred quail have higher corticosterone levels than non-transferred quail, that only seems to hold true for the H line quail, as A, L, and R lines all have non-transferred corticosterone values higher than transferred corticosterone concentrations (Figure 4).

Transfer

As stated before, transferred quail have higher levels of corticosterone (Figure 9) until bloodline is compared with transfer status. The corticosterone levels of the H line seem to be large enough to impact transfer status numbers when combined with the other lines so that it looks like the H line impacts the average of the other lines, which makes sense given that the high stress quail were genetically selected for high corticosterone response. In Figure 5, transferred quail tend to have higher corticosterone levels as the light intensity increases.

Sample time

General corticosterone levels were higher before the lights turned on, then dropped once the lights started, and gradually increased as the light intensity increased. Room 1 had overall higher corticosterone levels than room 2 (Figure 1).

Discussion

Maintaining low levels of corticosterone could be key to improving production, performance, welfare, growth rate and egg production. The vast majority of environmental factors have been researched thoroughly to find out what it takes to help birds grow faster, but the main research in lighting has been focused on light intensity (Raccoursier et al., 2019) and day length. What little research that has gone into light transitions has been conducted in fish (Ryu et al., 2019).

While short-term quantification of corticosterone can be done with sampling blood, long-term measurement of corticosterone can be done by measuring corticosterone stored in the feather (Bortolotti et al., 2008). In Bortolotti and colleagues' study, they found that corticosterone levels in molted feathers of partridges was measured with a methanol-based extraction technique and correlated with egg production recorded over time. Although the lines of quail were not able to be kept separate to measure egg production as a result of inadequate facilities, several anecdotal observations were noted for each room. Quail in room 1, which was subjected to traditional lighting, came into production quicker than room 2. While quail in room 2 took longer to come into lay and reach peak production, the hens in room 2 consistently laid 2-3x more eggs daily than room 1. If the high corticosterone levels caused decreased egg production, it could also cause problems with hatchability and growth later. Corticosterone has also been shown to impact embryonic survival and offspring growth (Peixoto et al., 2020).

Not only could the transferred quail have been impacted by the light systems changing, but by the handling and move itself. Handling stress response could be avoided with a remote blood sampling system like one created for turkeys (McMurtry & Brocht, 1984), but quail are small and this might not be the best option. Corticosterone has a half-life of 22 minutes in male broilers (Birrenkott & Wiggins, 1984) and 9.8 minutes in Coturnix quail (Kovács & Péczely, 1983) so it would not remain in the body for that long, but the stress induced during transport from room to room could have affected the birds' growth. Hull and colleagues (2007) found that young coturnix quail dosed with corticosterone had lower weights of the spleen, thymus, bursa, muscle, testes, and oviduct. While stressors and egg production tracked with egg charts is beyond the scope of this study, it merits further research.

Bird welfare continues to be at the forefront of the poultry industry, but it is usually measured by broken bones at the processing plant. Not only does corticosterone provide a good measure of welfare, but it can also provide insight into how to improve production and production practices. Since corticosterone can be traced both long-term and short-term, this could help producers fine tune their lighting practices and track changes within weeks.

Conclusions

Initial findings from this study suggest that sunrise/sunset lighting may be better for bird health and welfare than traditional on/off lighting through corticosterone response. This study also revealed that birds transferred between lighting programs were not affected. While room impacted corticosterone levels, going from dark to light did not. This research is the first step in determining new techniques regarding lighting that producers can utilize to help improve the health and welfare of their production flocks. Future research that expands upon this data and looks at a larger sample size of quail is imperative moving forward. Although it does appear that the stress response of lighting was not line specific, focusing on one single line of quail subjected to either on/off lighting or sunrise/sunset lighting is necessary in the future.

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Tables and Figures

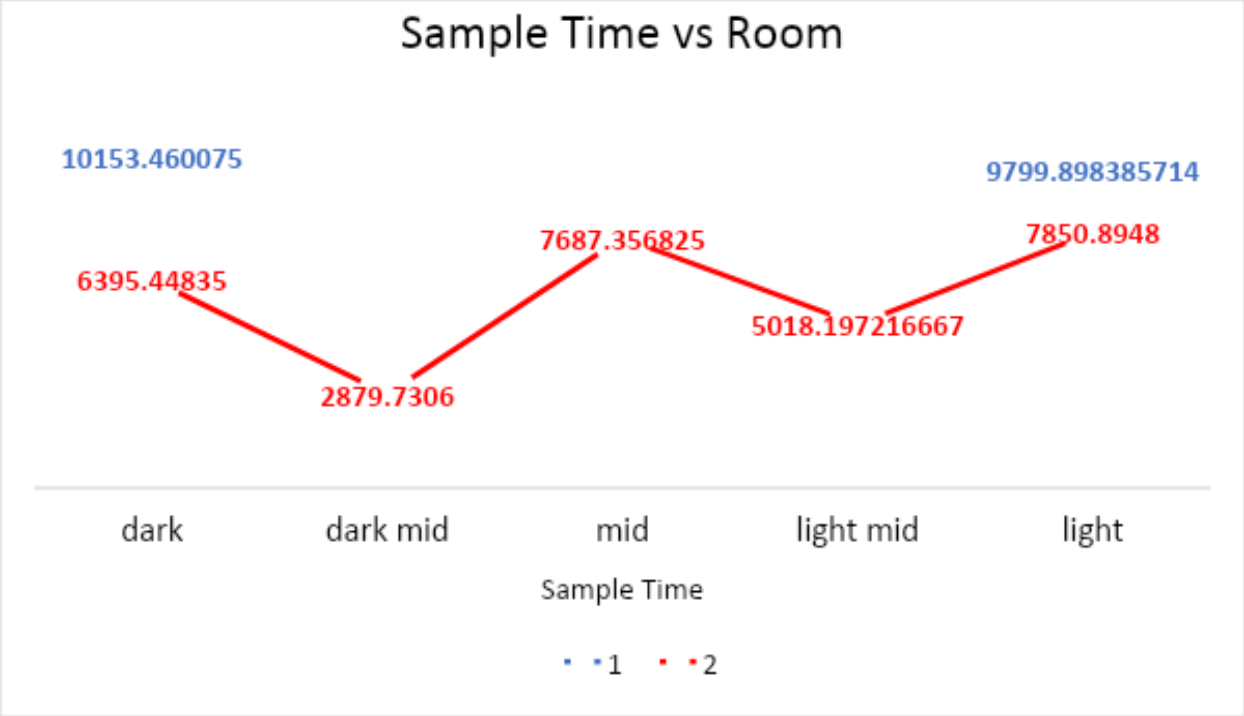
Table 1. Corticosterone concentrations in response to conditions

Room[1]	Sample time[2]	line[3]	transferred[4]	Cort (pg/mL)
1	dark	h	n	2457.9229
1	light	h	n	15053.295
1	dark	l	n	5165.3394
1	light	l	n	574.5028
1	dark	a	n	15053.295
1	light	a	n	8920.4084
1	light	a	y	1633.9266
1	dark	r	n	17937.283
1	light	r	n	397.9321
1	light	r	y	1951.7538
2	dark	h	n	562.7314
2	dark	h	n	5459.6239
2	dark	h	n	4564.9993
2	mid	h	n	4341.3431
2	light	h	n	4423.7428
2	dark	l	n	15406.437
2	light	l	n	6483.7336
2	light	l	n	15524.15
2	dark	a	n	5388.9956
2	dark	a	n	1221.9284
2	light	a	n	5165.3394
2	dark	r	n	4223.6294
2	dark	r	n	3682.1461
2	light	r	n	445.0176
2	light	r	n	3105.3486
2	mid	h	y	15347.58
2	light	h	y	5930.4789
2	light	l	y	2893.4639
2	light	l	y	11404.169
2	dark	a	y	2128.3244
2	mid	a	y	6460.1908
2	light	a	y	4423.7428
2	light	a	y	8637.8954
2	dark	r	y	221.3615
2	mid	r	y	4600.3134
2	light	r	y	10697.886
2	light	r	y	5930.4789

*Any holes in the data are from corticosterone levels too low to calculate

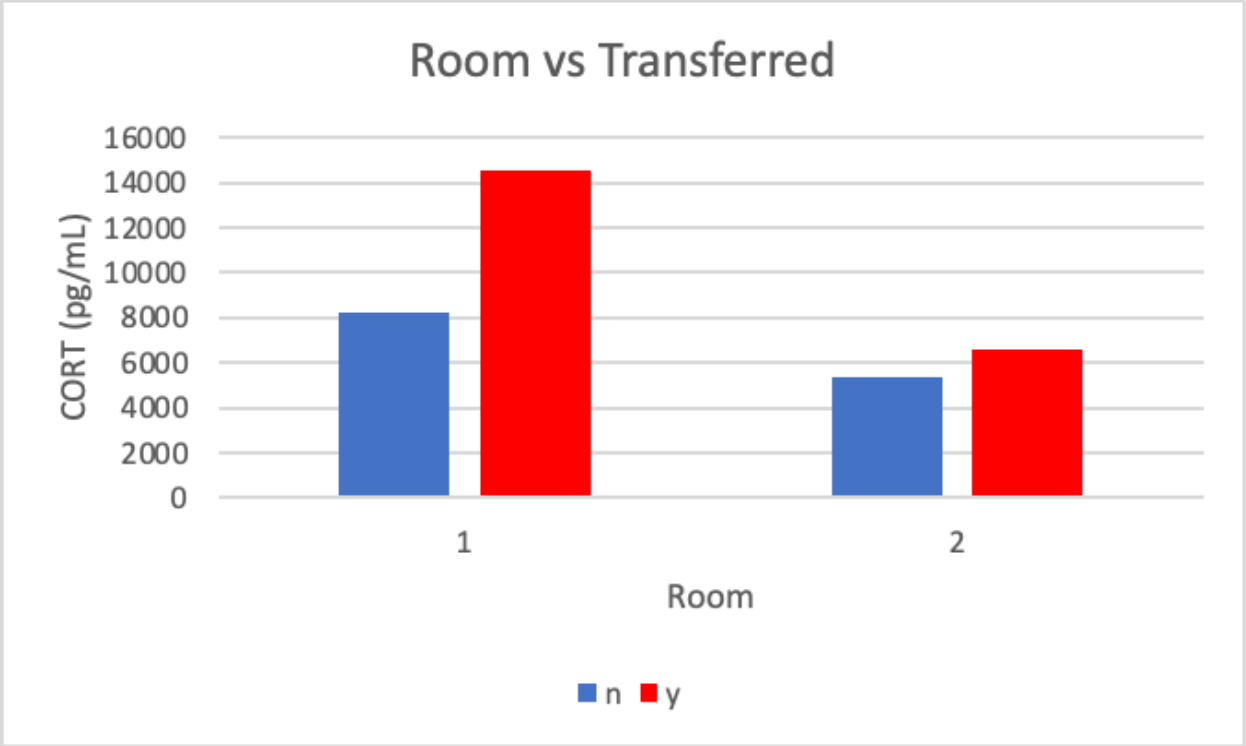
[1]-Room 1-standard on/off lighting, Room 2-sunrise/sunset lighting
[2]-dark-before lights on, mid-30 minutes into lights on (room 2), light-lights on at full intensity
[3]-A-AR random, H-high stress, L-low stress, R-random bred control
[4]-n=not transferred, y=transferred

Figure 1. CORT (pg/mL) room averages at each sample time .



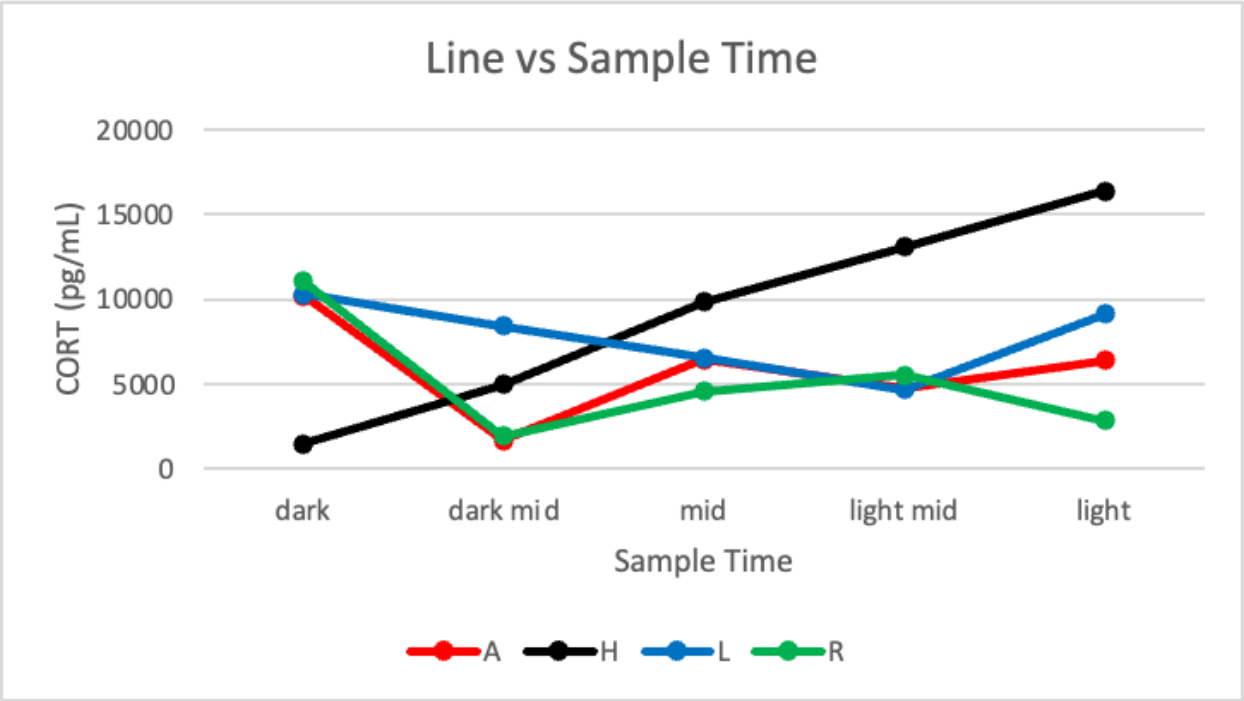
[1]-Room 1-standard on/off lighting, Room 2-sunrise/sunset lighting
[2]-dark-before lights on at 8:00, dark mid-20 min into lights on, mid-40 minutes into lights on, light mid-60 minutes into lights on, light-lights on at full intensity after 9:00

Figure 2. CORT (pg/mL) averages by room and transfer status



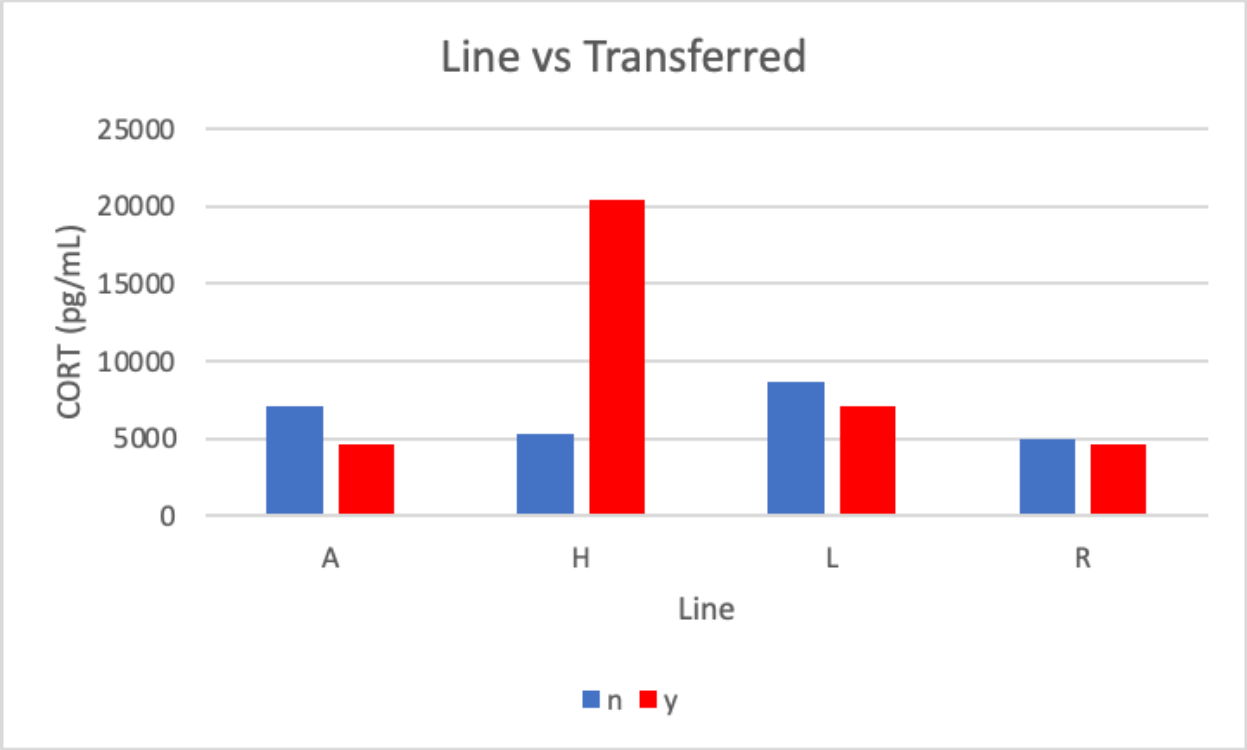
[1]-Room 1-standard on/off lighting, Room 2-sunrise/sunset lighting
[2]-n=not transferred, y=transferred

Figure 3. CORT (pg/mL) averages by line and sample time



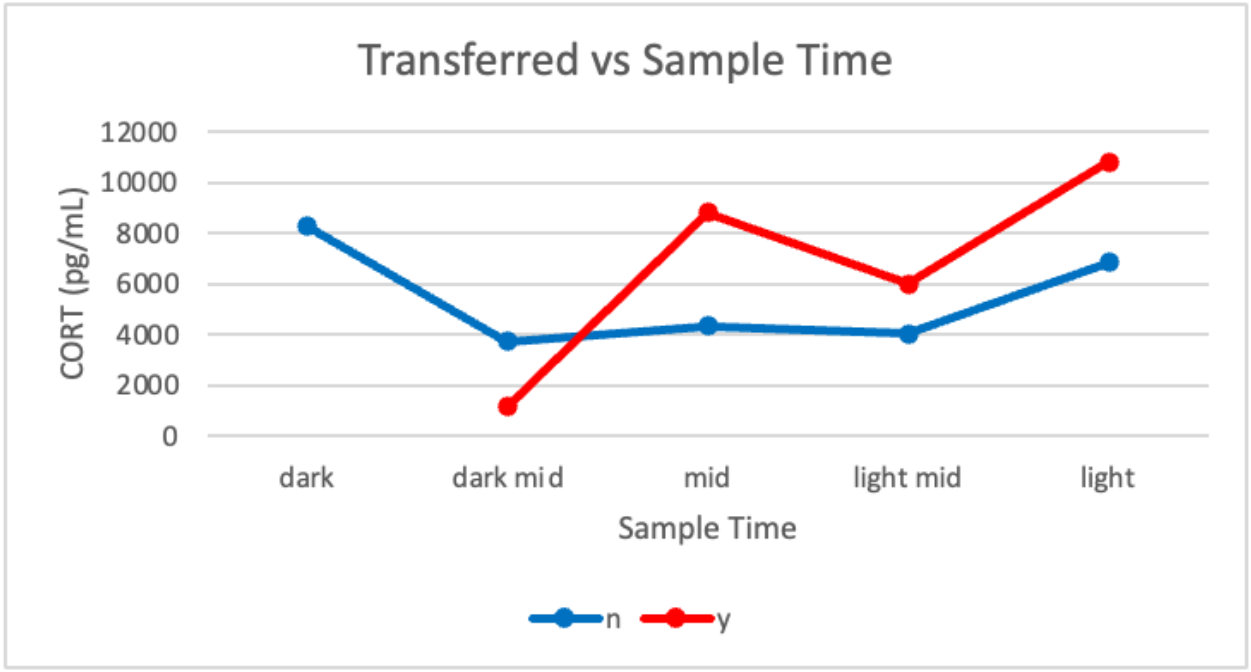
[1]-A=Arkansas Random, H=High Stress, L=Low Stress, R=Random
[2]-dark-before lights on, dark mid-20 min into lights on, mid-40 minutes into lights on, light mid-60 minutes into lights on, light-lights on at full intensity

Figure 4. CORT (pg/mL)-Line averages by transfer status



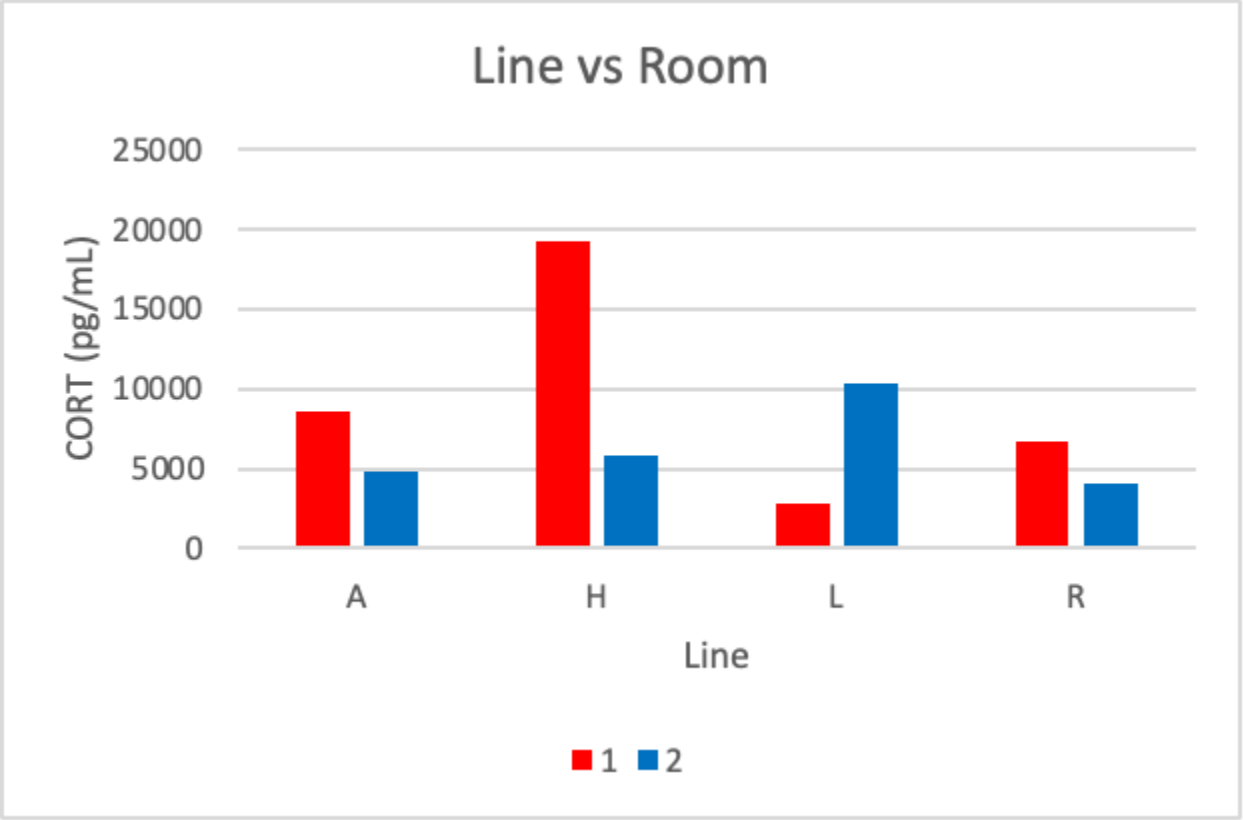
[1]-n=not transferred, y=transferred
[2]-A=Arkansas Random, H=High Stress, L=Low Stress, R=Random

Figure 5. CORT averages by transfer status and sample time



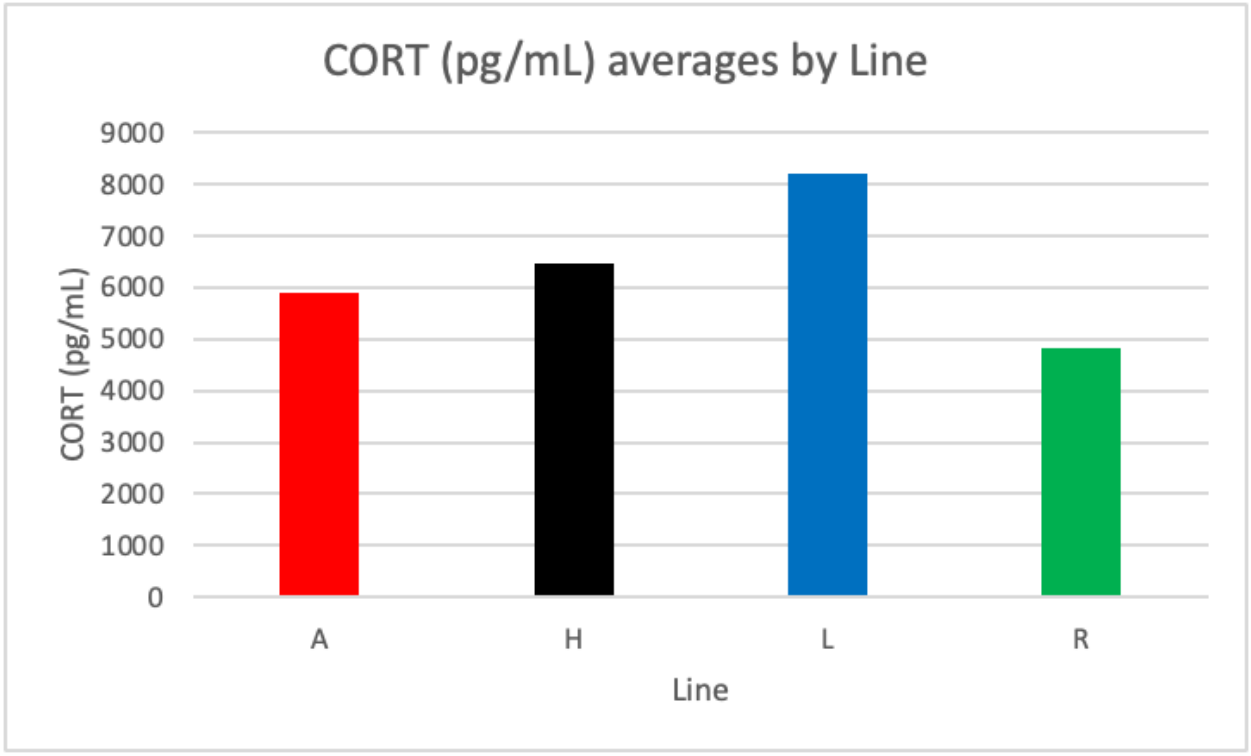
[1]-dark-before lights on, dark mid-20 min into lights on, mid-40 minutes into lights on, light mid-60 minutes into lights on, light-lights on at full intensity
[2]-n=not transferred, y=transferred

Figure 6. CORT (pg/mL) averages by line and environmental room



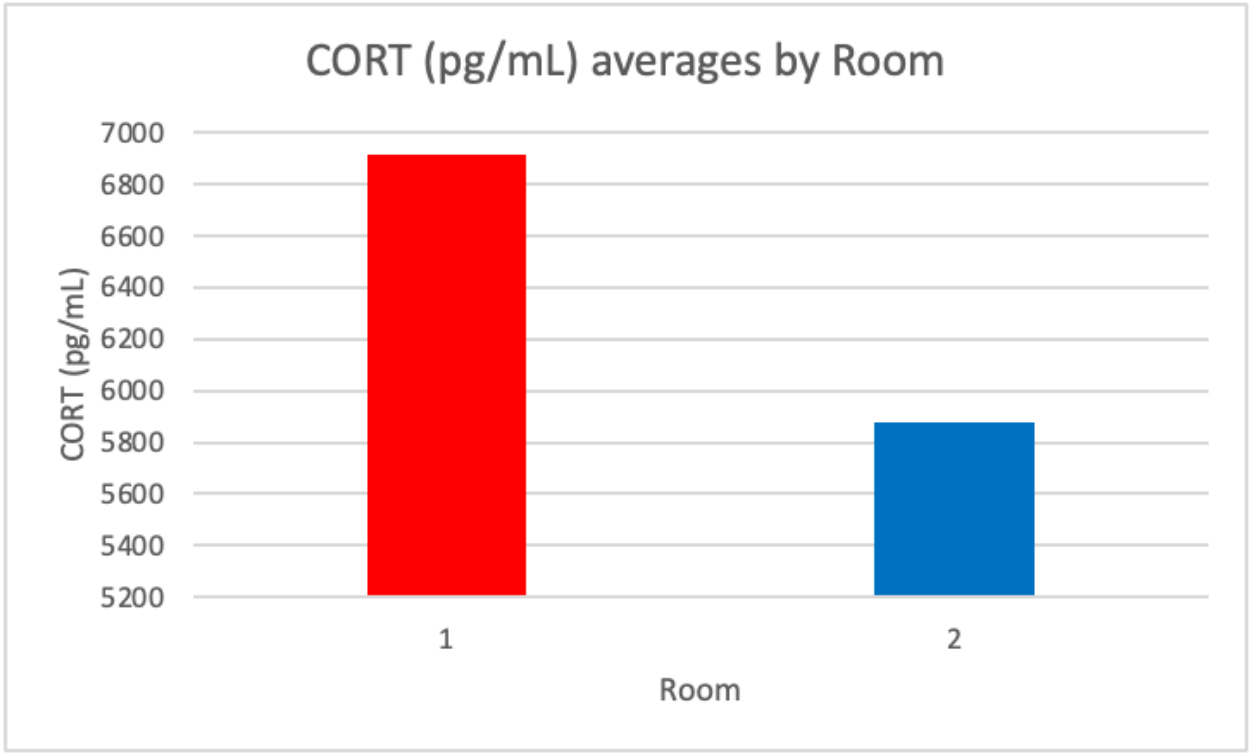
[1]-Room 1-standard on/off lighting, Room 2-sunrise/sunset lighting
[2]-A=Arkansas Random, H=High Stress, L=Low Stress, R=Random

Figure 7. CORT (pg/mL) averages by genetic line



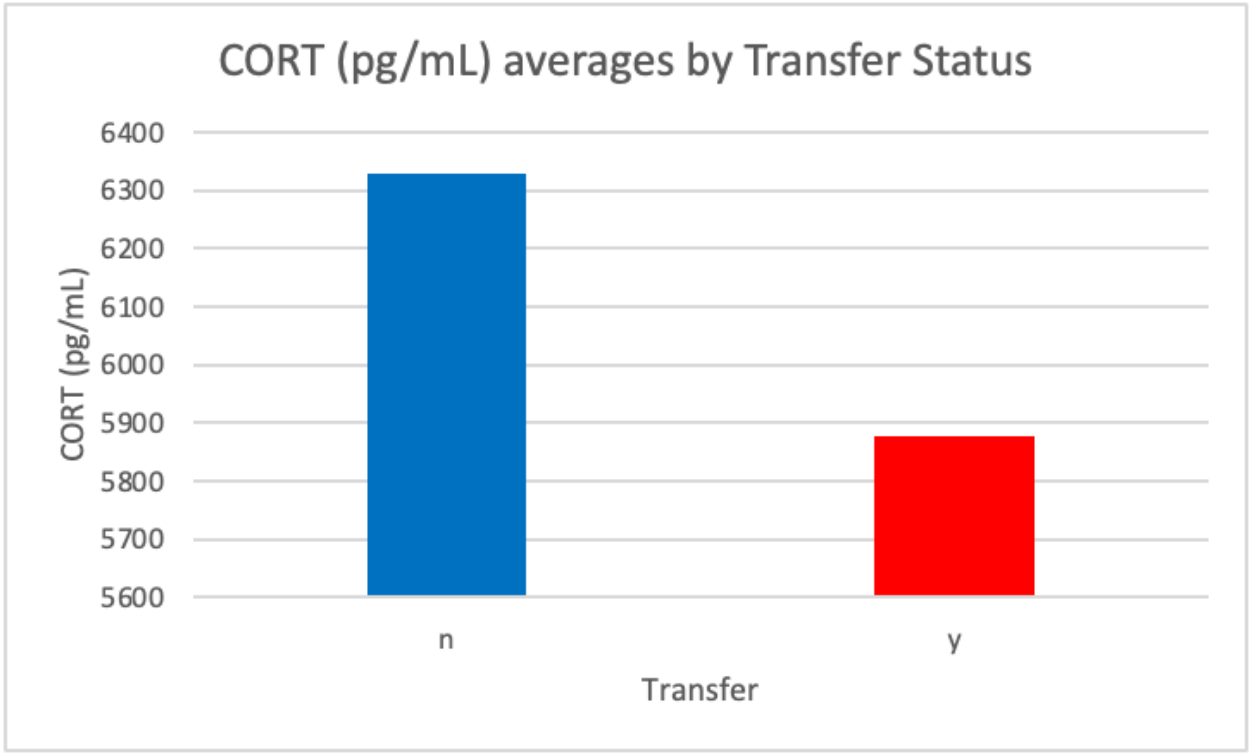
[1]-A=Arkansas Random, H=High Stress, L=Low Stress, R=Random

Figure 8. CORT (pg/mL) averages by room



[1]-Room 1-standard on/off lighting, Room 2-sunrise/sunset lighting

Figure 9. CORT (pg/mL) averages by transfer status



[1]-n=not transferred, y=transferred